A Microbiological Process Report

Enzymatic Hydrolysis of Cellulose

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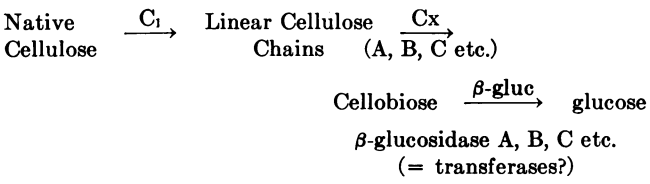
Enzymatic hydrolysis of cellulose is an important reaction in nature for it marks the first step in the decay of cellulose, the most abundantly occurring organic material. Unlike the chemical reactions, the enzymatic process has not yet been adapted to any useful industrial purpose.

This paper aims to review and to evaluate some of the recent developments in the study of the enzymatic hydrolysis of cellulose, to compare enzymatic with other hydrolyses, and to define the problems immediately ahead.

RECENT DEVELOPMENTS

Multiple components in cellulolytic systems. One of the most important questions being resolved is "Are there several cellulases, or is there only one type?" Until 4 or 5 years ago, all of our work was based on the assumption of a single cellulase, and this in spite of the fact that starch hydrolyzing enzymes were recognized as of several types.

The following diagram helps to illustrate the different views of the current workers in this field:



Whitaker (1953–1954) has concluded that his *Myrothecium verrucaria* cellulase is a single enzyme capable of hydrolyzing native cellulose to glucose. Kooiman *et al.* (1953) agree with Whitaker except that they involve a separate enzyme (cellobiase) in the hydrolysis of cellobiose to glucose. Jermyn (1952) has demonstrated the presence of several β-glucosidases, some of which he believes act on comparatively long chains as found in carboxymethylcellulose (CMC). We question this, since Grassmann *et al.* (1933) showed that six approaches the upper limit in the number of anhydroglucose units on which β-glucosidase (cellobiase) can act. Gilligan and Reese (1954) have separated by paper chromatography and with calcium phosphate columns, several cellulolytic components (Cx) from filtrates of the same species (*M. verrucaria*) used by these workers. Fractions from *Trichoderma viride* filtrates differed from each other in their rate of movement on the column, in their relative activities on certain cellulosic substrates, in their mode of action, and in their behavior in the presence of cellobiose and of methocel (table 1). In addition, there was a marked synergistic effect shown when fractions were recombined and tested on solid celluloses (table 2).

Until now, the rate of enzyme hydrolysis of cellulose was believed to be independent of the degree of polymerization (DP). Separation of *Trichoderma viride* cellulase into three components shows that while this may be true of whole filtrates, it is *not* true of the individual components. Of the three *T. viride* components, one (B), was more active on longer chains, one (CD), on the shorter chains, and the third (A), about equally active on both (table 1). This relationship suggests that the components of a cellulolytic system may differ largely in their preference for chains of a particular length. If this be true, then there may be components bridging the gap between the cellulases and the β-glucosidases (Jermyn, 1952). Such components would have maximum activity on the short chains (6 to 10 units long) and lesser activity as the chains become longer or shorter. There is another explanation, however. The low DP material, by its very nature, has ten times as many chain ends. The preference of enzyme component CD for this material may thus be an indication of an endwise attack, rather than a preference for short chains. Improved separation of components is necessary before this question can be resolved.

Not enough is known about C_1 to characterize it. The existence of this enzyme was proposed at a stage in our development when Cx was considered to be a single enzyme. Its presence was deduced from the fact that some organisms unable to attack native cellulose did produce enzymes capable of hydrolyzing degraded celluloses (like CMC). Now that we have found differences in Cxs, we are uncertain whether C_1 is that form of Cx acting primarily on the longest chains, or whether it may indeed act on some minor linkage, or minor component, in native cellulose. In this connection, we call attention to the peculiar action of one

TABLE 1. *Comparison of various enzyme fractions obtained by passing Trichoderma viride filtrate through a calcium phosphate gel column*

Fraction	R* W/C	R* SF/C	Inhibition† of CMC Hydrolysis by Cellobiose and Methocel					Ratio of Activity on Cellulose of DP‡ 480 to Activity on Cellulose of DP 46
			Cellobiose Conc.			Methocel Conc.		
			0.5	1.0	2.0	0.006	0.02	
			%	%	%	%	%	
A	0.48	13.0	+3.0	+5.0	2.0	(+4)	0	1.1
B	0.34	1.1	25.0	36.0	42.0	35.0	64.0	1.6
CD	12.0	11.0	27.0	NT	51.0	33.0	63.0	0.66
Original	1.5	23.0	21.0	32.0	34.0	36.0	41.0	

* R values are comparative values for the action of the enzyme on two substrates. These substrates are: W = Walseth cellulose, i.e. reptd. from 85% H_3PO_4 (Gilligan and Reese, 1954). C = CMC 50T Degree of Substitution = 0.52 (Gilligan and Reese, 1954). SF = Swelling factor, an activity against cotton measured by the increase in swollen weight in 18% NaOH (Marsh *et al.*, 1953).
† Values in ( ) are probably not significant; NT = No Test; + = Stimulation. These are results obtained in viscosity tests.
‡ DP = Degree of polymerization.

cellulolytic component on cotton fiber, for example, the swelling factor. Short incubation of fiber with this enzyme increases the subsequent swelling in 18 per cent alkali and increases the Congo red uptake (Marsh *et al.*, 1953). The action appears to be on the cellulose of the primary wall. Since no reducing sugars are produced under the test conditions, it may be that the enzyme involved is limited in its action to the long chains.

Intermediate products of cellulose hydrolysis. A second problem receiving increased emphasis is "What are the products of the enzymatic hydrolysis of cellulose?"

Cellulase acts on cellulose to produce soluble sugars. Intermediate hydrolysis products are never found. This indicates that the total action is such that the initial attack is slow relative to the later reactions. As a result, the degree of polymerization of the residue falls rather slowly (Walseth, 1952), as compared to acid hydrolysis. Our results, based on a different measure of DP (end group, Meyer, 1951), confirm and extend those of Walseth.

Cotton DP 519 —(10% NaOH, 2.2% soluble)→ Cotton DP 752

↓ Enzyme, 0.6% Loss

Cotton DP 407 —(10% NaOH, 4.1% soluble)→ Cotton DP 850

The increase in alkali solubility due to enzyme action was previously reported by Abrams (1950). It seems that native cotton has cellulose chains of variable length. The shorter of these are removed by treatment with cold alkali, leaving a residue of higher DP. Cellulase appears to act preferentially on the shorter chains. As a result, the alkali soluble fraction increases, and the DP of the residue reaches a value even higher than that of the initial material.

Glucose is certainly the end product of the complete hydrolysis of cellulose by enzymes Where β-glucosidase is present in the cellulolytic complex, much of the glucose results from its action on cellobiose. But it is still uncertain whether glucose may be produced in the absence of β-glucosidase. Whitaker (1954) believes that it can. Some of our own recent data (figure 1) indicate

TABLE 2. *Effects on combination of cellulolytic fractions from calcium phosphate gel columns*

Filtrates	Fractions	Relative Activities*		
		Walseth	SF	Cx
Trichoderma viride QM 6a	A	1.0	1.0	1.0
	CD	1.0	1.0	1.0
	(A + CD)/2	2.1	2.0	1.0 ± 0.06
Myrothecium verrucaria QM 460	First	1.0	1.0	1.0
	Last	1.0	1.0	1.0
	(First + last)/2	1.9	2.3	1.0–1.06

* For activities, see footnote to table 1.

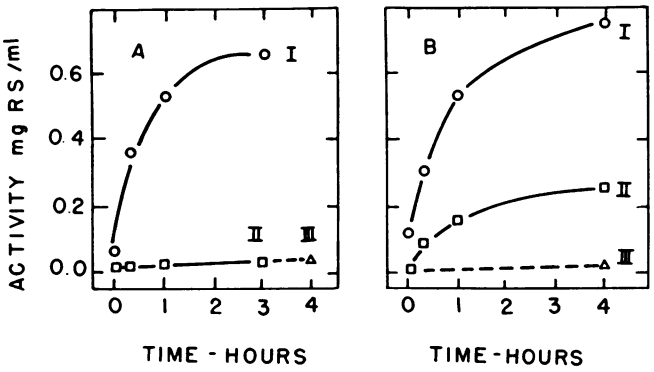


FIG. 1. Products of enzymatic hydrolysis of cellulose. Activity given as mg reducing sugar (RS) per ml of reaction mixture.

A. *Myrothecium verrucia.*
B. *Trichoderma viride.*
I. ○———○ Cellobiose from "Walseth" cellulose (0.25%).
II. □———□ Glucose from "Walseth" cellulose (0.25%).
III. △- - - -△ Glucose from cellobiose (0.25%) (under same conditions as used in cellulose hydrolysis).

that glucose does result from direct action of Cx (or of a single component of Cx). The solutions selected have low cellobiase activity. The *M. verrucaria* solution (a fraction obtained by alcohol precipitation) produced but a trace of glucose from cellulose, and this could be due to the cellobiase present. Here, it appears that cellobiose is the only end product of Cx activity. The *T. viride* filtrate, on the other hand, produced an appreciable amount of glucose which must come from direct action of Cx on cellulose, since direct action of the filtrate on cellobiose produced only a trace of glucose. Indeed, study of components relatively free of cellobiase makes it appear that this is the more common action. A cellobiose:glucose ratio of 2 (and of 3) has been found in hydrolysates of the latter type.

We have never found a water soluble intermediate product of cellulose hydrolysis (that is, other than cellobiose). The only exceptions have been those attributable to substituted sugars resulting from hydrolysis of CMC, cellulose sulfate, or other substituted celluloses (Levinson *et al.*, 1951). "Intermediates" were detected by Kooiman *et al.* (1953) during the enzymatic hydrolysis of cellulose dextrin (obtained by treating cellulose with strong sulfuric acid). We have also shown such intermediates by paper chromatography (Levinson *et al.*, 1951), but believe them to be substituted sugars. Recently, Hash and King (1954) have demonstrated what they believe to be a 4-unit (soluble) intermediate. In view of recent observations that transferases are present in these solutions (Crook and Stone, 1953), it is probable that the intermediate observed by Hash and King may be the result of the action of such a transferase on cellobiose.

$$\text{Cellobiose} + \text{Cellobiose} \xrightarrow{\text{glucotransferase}} \underset{\text{glucose}}{\overset{\text{cellotriose}}{\longrightarrow}} \text{glucose}$$

$$\text{G-1} \overset{\beta}{\text{—}} \text{4-G} + \text{G-1} \overset{\beta}{\text{—}} \text{4-G} \xrightarrow[\text{transferase}]{\text{gluco}} \text{Cellobiose}$$

$$\text{G-1} \overset{\beta}{\text{—}} [?] \text{ G-1} \overset{\beta}{\text{—}} \text{4-G} + \text{G}$$

trimer glucose

This reaction has been observed not only by Crook and Stone (1953), but in cellulolytic filtrates of *Streptomyces sp.* (QM B814) by B. Norkrans (*personal communication*), of *Myrothecium verrucaria* (QM 460), *Trichoderma viride* (QM 6a), *Pestalotia palmarum* (QM 381), *Stachybotrys atra* (QM 94d) by E. T. Reese (*unpublished*); and in noncellulolytic filtrates of *Aspergillus luchuensis* (QM 873) by E. T. Reese (*unpublished*), of *Aspergillus niger* by Barker *et al.* (1953), of *Aspergillus flavus* by Giri *et al.* (1954), and of *Aspergillus oryzae* by Jermyn and Thomas (1953). The transferases thus appear to be of common occurrence, and it may be that all β-glucosidases act in this fashion.

The nature of the products of transferase activity needs to be clarified. Barker *et al.* (1953) believe that three cellotrioses are produced, the major trimer containing β 1–6 and β 1–4 linkages (gentiobiose and cellobiose were produced on acid hydrolysis). Disaccharides other than cellobiose are also found. As shown by Barker *et al.* (1953), these may be the result of action of a transferase on glucose, or they may result from hydrolysis of the various trimers. In all cases, the products were said to contain β-linkages, though not necessarily of the 1–4 type.

We have found that the above listed cellulolytic filtrates which produce glucose and oligosaccharide(s) from cellobiose, also produce glucose and an oligosaccharide from maltose, a disaccharide with an α linkage. It appears that filtrates of fungi may contain some transferases acting on α linked, and others acting on β linked disaccharides. While the α type transferase presumably does not act on cellobiose, it may be responsible for the production of α linkages in saccharides produced from the glucose resulting from the β type transferase. Most of the reports to date appear to disregard the effect of contaminating enzymes on the nature of the resultant products. The variety of the products suggests the possibility that several enzymes are involved and that, until separation of the enzymes is achieved, the part played by each will be open to question (Jermyn and Thomas, 1953).

Purification and characterization of cellulase. The best work on the purification of cellulase is being done by Whitaker (1953) in Canada. Using modern methods, he has succeeded in obtaining a highly purified preparation which he has proceeded to characterize. According to him, the cellulase of *Myrothecium verrucaria* appears to be a cigar-shaped molecule, roughly 200 Å long by 33 Å broad, having a molecular weight of about 63,000 (Whitaker *et al.*, 1954). As Cooke (1954) has pointed out, such a large molecule is incapable of penetrating the pores of cotton or rayon. Whitaker's preparation hydrolyzes cellulose and its breakdown products of all chain lengths, though the rate falls off markedly as the DP falls from four to two.

Whistler and Smart (1953) have removed the cellobiase from a commercial cellulase preparation. As a result, cellobiose was the main product of hydrolysis by the purified cellulase preparation. The best work of this group has been the development of a chromatographic technique for separating the sugars found in hydrolysates.

The resistance of cellulase (of *M. verrucaria*) to heat inactivation (Bultman and Leonard, 1954; Kooiman *et al.*, 1953) has been used to study the hydrolysis of cellulose in the absence of β-glucosidase (which is more sensitive to heat).

TABLE 3. *Comparison of enzyme and acid hydrolysis of cellulose*

Substrate	Amount of Agent to Give Unit Activity*	
	Trichoderma viride enzyme†	Hydrochloric acid
	ppm	*ppm*
Carboxymethylcellulose (C)	2	>200,000
"Walseth" cellulose (W)	6	>200,000
Cotton fibers	1	64,000

* Concentration required to give unit activities: C = 0.40 mg reducing sugar/ml/hr/ 50 C. W = 0.50 mg reducing sugar/ml/2hr 50 C. Cotton = 50 mg increase in swollen wt/hr 50 C.

† Derived from data of Gilligan and Reese (1954).

COMPARISON OF ENZYMATIC WITH OTHER HYDROLYSES

Specificity of cellulolytic enzymes. Enzymes are more efficient agents of hydrolysis than are acids (table 3). A comparison of activities at 50 C on three cellulosic substrates shows that 100,000 times as much acid is required to bring about the same degree of hydrolysis. At the molecular level, the difference is further increased because of the disparity in molecular weight [HCl = 36; cellulase = 63,000 (Whitaker, 1954)], so that approximately 10^8 HCl molecules are required to do the work of a single enzyme molecule under the conditions specified.

Hydrolysis by enzymes is a highly specific reaction. Acid attacks α or β and 1 to 4, 1 to 6, 1 to 2, or 1 to 3 linkages. Known cellulolytic enzymes hydrolyze only the β 1 to 4 glucosidic link. The specificity of cellulases extends even beyond this, for if the glucose units of the chain are substituted or modified in any way, the action of the enzyme is impaired. As a result, treatment of an unknown glucosan with a known enzyme solution has become a feasible method for determining the nature of the linkages involved. As yet, this technique is not highly developed. But as the components of enzyme systems are separated, and as better characterized substrates are developed, we may expect to obtain a tool for the further elucidation of carbohydrate structure.

In spite of the great activity of cellulase, we are unable to degrade cellulose rapidly by enzymatic means. We know of no enzyme that can compete with the sulfuric acid hydrolysis of native cellulose to glucose by the method of Saeman *et al.* (1944), practically complete conversion within one hour. But this requires the combined action of concentrated acid at low temperature and of dilute acid at high. It involves a swelling and a splitting (and perhaps much more!). While we cannot use cellulolytic enzymes to achieve rapid extensive dissolution of native cellulose, the organism itself can do much better than we can. Highly active organisms in shake flasks can consume over 50 per cent of the cellulose (0.5 per cent suspension) within 3 or 4 days. Yet if we were to use the same solution, free of the organism, taken at the time of its greatest cellulolytic activity and adjusted to conditions for maximum hydrolysis (that is, higher temperatures than the growth optimum, and perhaps a different pH from that giving best growth), we achieve only a fraction of the rate attained by the growing organism. The reason may lie in the fact that in the absence of the organism the end products accumulate, and of these at least cellobiose is known to be inhibitory (Reese *et al.*, 1952). Or it may be that all of the cellulolytic enzymes are not found in the culture filtrate. Daily replenishing of the solution with fresh enzyme solution helps but little. It is more likely that the intimate association of hypha with cellulose accounts for the greater hydrolysis rate in the growing culture.

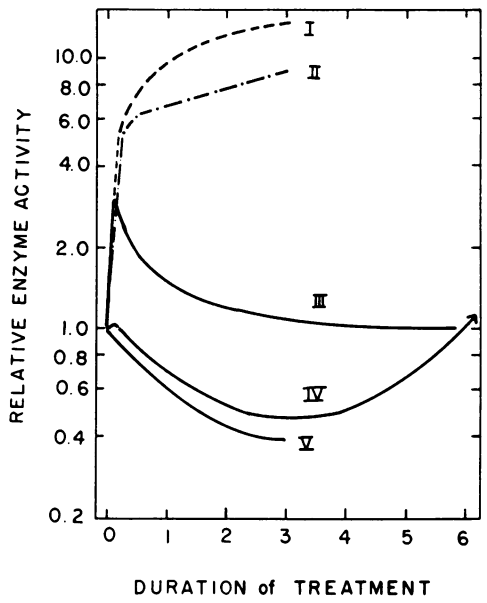


FIG. 2. Effect of various treatments on the susceptibility of cotton cellulose to enzymic hydrolysis.

I. Effect of 85 per cent H_3PO_4; or of 72 per cent H_2SO_4. Abscissa in hours.
II. Effect of ball milling (vibratory). Abscissa in hours.
III. Effect of 30 per cent NaOH. Abscissa in days exposure of alkali swollen material to air.
IV. Effect of irradiation with cathode rays (van de Graaf). Abscissa in tens of megareps.
V. Effect of weak organic acids. Abscissa in days of refluxing. (Loss in weight = 5 to 6 per cent in 3 days.)

There are two important ways to increase the rate of enzyme hydrolysis. One is to give the enzyme the conditions which are optimal for it. The other is to alter the cellulose in such a way as to attain maximum surface. Solubilization by the addition of substituents is ideal if the degree of substitution (DS) can be kept low (below 0.5). Above DS 1, the cellulose becomes resistant to enzyme action.

Changes in the physical or chemical nature of cellulose lead to changes in its susceptibility to enzyme hydrolysis (figure 2). Swelling by strong acid (72 per cent sulfuric or 85 per cent phosphoric) or by strong

alkali increases the susceptibility of the reprecipitated product to enzyme hydrolysis. Phosphoric acid treatment is preferable for most purposes, since it does not involve partial esterification of the cellulose as does the sulfuric acid treatment. Exposure of alkali cellulose to air leads to changes making the swollen cellulose more resistant to enzyme hydrolysis. *Mechanical* increase in the surface results in a greatly increased susceptibility to enzyme hydrolysis. Partial disintegration in a Wiley Mill has a marked effect which is apparently greater than can be accounted for by the increase in surface. It appears that the cut surfaces are much less resistant than the outer layers of the cotton fiber. Ball milling (figure 2) gives an even greater effect, due in part to the increase of surface and in part to a depolymerization.

Treatment of native cellulose with dilute mineral acid, or with weak organic acids, reduces the susceptibility of the residual cellulose to enzyme, either by the removal of the more readily hydrolyzed (amorphous) areas or by the formation of substituted cellulose derivatives. While marked loss in tensile strength of cotton, and a lower DP results from the treatment with dilute mineral acid, there is no increase in susceptibility to enzyme hydrolysis.

Irradiation with cathode rays modifies cellulose in such a way as to make it more resistant to enzyme hydrolysis. Ultraviolet light seems to have a similar effect (Wagner *et al.*, 1947). Heavy dosages of cathode rays bring about a reversal in effect (at about 40 megareps), the residue increasing again in susceptibility to enzyme action. At high dosage levels, depolymerization becomes an important factor. Its influence is apparently sufficient to outweigh the other changes being produced along the cellulose chain.

Although the effects of the various treatments are in general the same for filtrates of different organisms, they are not of the same magnitude (table 4). The cellodextrin, for example, is hydrolyzed 21 times as fast as linter cellulose by *Myrothecium verrucaria* filtrate, but only 7 times as fast by *Aspergillus fumigatus*. Solka Floc (a purified wood cellulose) is hydrolyzed 4 times as fast by filtrates of *T. viride* as by those of *M. verrucaria* (figure 3A), but CMC and Walseth cellulose are more susceptible to the *M. verrucaria* filtrates. The relative susceptibilities of various substrates to enzyme hydrolysis have been used to characterize these enzymes and to aid in identification of the components of enzyme complexes. Another difference between filtrates is in the moisture regain values of residues from enzyme hydrolysis (table 5). These do not bear the expected relationship to the per cent loss during hydrolysis. Other differences in the action of filtrates from various sources with respect to differences in adsorptive properties, in temperature and pH optima, have been reported previously.

Most current investigators have chosen *M. verrucaria* for their study of cellulolytic enzymes (Bultman and Leonard, 1954; Crook and Stone, 1953; Hash and King, 1954; Kooiman *et al.*, 1953; and Whitaker, 1953).

TABLE 4. *Comparative action of enzymes of different organisms on cellulosic substrates*

Substrate	*Aspergillus fumigatus*	*Streptomyces* sp.	*Myrothecium verrucaria*
Cellodextrin from linter cellulose	67*	170	210
Alkali cellulose from linter cellulose	17	40	42
Linter cellulose	10	10	10
Cotton sliver, ground (40 mesh)	6	11	6
Solka Floc (purified wood cellulose)	5	8	5
Cotton silver, unground	3	1	0

* These relative values are obtained by setting the reducing sugar value of the linter cellulose hydrolysate at 10. A value of 67 indicates that 6.7 times as much sugar was produced from cellodextrin as from the linter cellulose, under similar conditions.

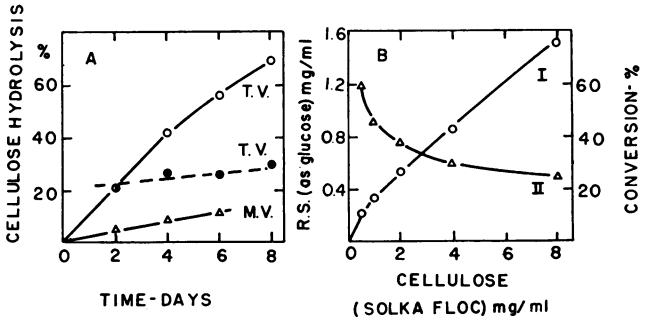


FIG. 3. Hydrolysis of cellulose (Solka Floc)

A. Effect of time, and of enzyme source. Enzyme solution changed every second day. Temperature ca 25 C, reciprocal shaker. Solka Floc concentration = 4 mg/ml. T.v. = *Trichoderma viride* filtrate pH 4.5. M.v. = *Myrothecium verrucaria* filtrate pH 5.0. Solid lines = cumulative values. Dotted lines = per cent hydrolysis of the substrate present at the beginning of each two-day period.

B. Effect of cellulose concentration on total production of sugars, and on per cent conversion of cellulose to sugars. *Trichoderma viride* filtrate pH 4.5 25 C reciprocal shaker. Time 24 hrs. I. Production of reducing sugars (all glucose). II. Conversion of cellulose to glucose.

TABLE 5. *Moisture regain values of residues from enzyme hydrolysis*

Filtrate	Per cent Hydrolysis	Moisture Regain on Residue
		%
Buffer	0 (63 hours)	8.70
Trichoderma viride QM 6a	15 (63 hours)	8.19
Myrothecium verrucaria QM 460	8 (63 and 72 hours)	7.56

This is not a particularly fortunate choice. *Trichoderma viride* (QM 6a) produces filtrates which are much more active than those of *M. verrucaria* against native cellulose, while filtrates of *Penicillium pusillum* (QM 137g) can be obtained which are at least 10 times as active as those of *M. verrucaria* against such substrates as CMC. Our own preference at this time is *T. viride*.

Some experimental data on the extent of enzyme hydrolysis of native cellulose will show the limitations of the enzymatic process (figure 3B). With an unconcentrated filtrate of *T. viride* (one of our most active organisms), a substrate concentration (Solka Floc) of 16 mg/ml yields 1.5 to 1.8 mg of glucose per ml in 24 hours (10 per cent conversion). This is about the best yield that we can get from native cellulose. If maximum per cent conversion of cellulose to glucose is the goal desired, the conditions are reversed, that is, low concentrations of cellulose (figure 3B) must be used.

It is interesting to compare these yields with those obtained by Kitts and Underkofler (1954) using rumen organisms in an anaerobic fermentation. Unlike aerobic fungi, these rumen organisms do not liberate cellulase into the medium. Using the whole cultures to which an inhibitor has been added, the yields of glucose obtained are of a magnitude of 2.0 to 3.0 mg/ml, under conditions comparable to those with *T. viride* (above). Using enzyme extracted from the rumen bacteria, the glucose yields from cellulose are very low, for example, 0.2 mg/ml/24 hrs.

When cellulose (Solka Floc) is hydrolyzed over longer periods, replenishing the enzyme solution at two-day intervals, the rate of hydrolysis remains high (figure 3A). In fact, based on the amount of substrate present, the rates follow a gradually ascending curve. Too much weight should not be placed upon this rise, however, in as much as (figure 3B) the per cent conversion is an inverse function of the substrate concentration (which was becoming less at each replacement of solution). The data tend to indicate that the substrate is essentially homogeneous. Similar results were obtained using cotton (ball milled) as the substrate.

Modification of the cellulose can lead to a much more rapid production of reducing sugars by enzymes. The maximum yields have been 10 mg of glucose per ml per day from a 13 mg/ml suspension of Walseth cellulose (with maximum hydrolysis within the first few hours). It would appear that for any practical application involving the production of sugar, a preliminary chemical modification of the cellulose will be necessary.

Future Investigations

Increasing evidence of the complexity of the cellulase system requires that better methods be developed for the separation of closely related enzymes. Differences in adsorptive properties, in electrical charge, in solubility may all need to be employed to effect the desired separation. Once the components have been isolated, better means of characterizing them will have to be developed. Current knowledge of the physical nature of cellulose is rapidly increasing, but the chemical composition seems to be taken too much for granted. In particular what is required is a thorough investigation of minor components, and of minor deviations from the normal composition. Admittedly this is a difficult field. Rarely occurring linkages are hard to find. If β linkages are found occasionally in starch, why not a few α linkages in cellulose?

The enzymatic synthesis of dextran and of other polysaccharides will stimulate attempts to synthesize cellulose. Some of the work with the glucotransferases may have been aimed in this direction. We know almost nothing about cellulose synthesis in plants. A concerted attack on the problem, similar to that on dextran, would undoubtedly bear fruit. Unfortunately cellulose is so inexpensive that the incentive for such a program is lacking.

Sometimes we go to the newspapers for our predictions. According to a report in the *New York Times*, by the year 2000, wood will be used only for food. If true, an appreciable role in the conversion will belong to microorganisms. The present use of yeasts in converting sulfite pulping waste into food seems to be expanding. Cellulose, too, can be converted into fungus tissue (at about a 50 per cent efficiency). But can such material be made appetizing to humans, that is, as an appreciable part of the diet? Or must we lose another sizeable fraction of the food value by converting it to animal tissue? The human diet depends heavily on starches. Can we convert cellulose into starch, or a starchlike food? If we can, then perhaps the prediction of the *Times* will come true.

Acknowledgment

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References

Abrams, E. 1950 Microbiological deterioration of cellulose during the first 72 hours of attack. Textile Research J., **20(2)**, 71-86.

Barker, S. A., Bourne, E. J., and Stacey, M. 1953 Synthesis of β-linked glucosaccharides by *Aspergillus niger* (Strain 152). Chem. and Ind., **1953**, 1287.

Bultman, J. D., and Leonard, J. M. 1954 Investigation of cellulase of *Myrothecium verrucaria*. Naval Research Lab. Rept. 4423.

Cooke, T. F. 1954 Resistance to microbiological deterioration of resin-treated cellulosic fabrics. Textile Research J., **24**, 197-209.

Crook, E. M., and Stone, B. A. 1953 Formation of oligosaccharides during the enzymic hydrolysis of β-glucosides. Biochem. J., **55**, XXV.

Gilligan, W., and Reese, E. T. 1954 Evidence for multiple

components in microbial cellulases. Can. J. Microbiol., **1**, 90–107.

GIRI, K. V., NIGAM, V. N., AND SRINIVASAN, K. S. 1954 Synthesis of oligosaccharides during enzymic hydrolysis of cellobiose by *Aspergillus flavus*. Nature, **173**, 953–954.

GRASSMANN, W., ZECHMEISTER, L., TOTH, G., AND STADLER, R. 1933 Enzymatic splitting of polysaccharides. Ann. Chem., Justus Liebigs, **503**, 167–179.

HASH, J. H., AND KING, K. W. 1954 Demonstration of an oligosaccharide intermediate in the enzymatic hydrolysis of cellulose. Science, **120**, 1033–1035.

JERMYN, M. A. 1952 Fungal cellulases. II. Complexity of enzymes from *Aspergillus oryzae* that split β-glucosidic linkages, and their partial separation. Australian J. Sci. Research, Ser. B, **5**, 433–443.

JERMYN, M. A., AND THOMAS, R. 1953 Transferase activity of β-glucosidases of *Aspergillus oryzae*. Australian J. Biol. Sci., **6**, 70–76.

KITTS, W. D., AND UNDERKOFLER, L. A. 1954 Digestion by rumen microorganisms. J. Agr. and Food Chem., **2(12)**, 639–645.

KOOIMAN, P., ROELOFSEN, P. A., AND SWEERIS, S. 1953 Some properties of cellulase from *Myrothecium verrucaria*. Enzymologia, **16**, 237–246.

LEVINSON, H. S., MANDELS, G. R., AND REESE, E. T. 1951 Products of hydrolysis of cellulose and its derivatives. Arch. Biochem. and Biophys., **31(3)**, 351–365.

MARSH, P. B., MEROLA, G. V., AND SIMPSON, M. E. 1953 An alkali swelling test applied to cotton fiber. Textile Research J., **23**, 831–841.

MEYER, K. H. 1951 Present status of starch chemistry. Advances in Enzymol., **12**, 341–377.

REESE, E. T., NORKRANS, B., AND GILLIGAN, W. 1952 Effect of cellobiose on the enzymatic hydrolysis of cellulose and its derivatives. Physiol. Plantarum, **5**, 379–390.

SAEMAN, J. F., BUBL, J. L., AND HARRIS, E. E. 1944 Quantitative saccharification of wood and cellulose. U.S.D.A. Forest Service, Forest Products Lab. Bul. R1458, Madison, Wisconsin.

WAGNER, R. P., WEBBER, H. H., AND SIU, R. G. H. 1947 Effect of ultraviolet light on cotton cellulose and its influence on subsequent degradation by microorganisms. Arch. Biochem., **12**, 35–50.

WALSETH, C. S. 1952 Influence of fine structure of cellulose on the action of cellulases. Tappi, **35**, 233–238.

WHISTLER, R. L., AND SMART, C. L. 1953 Isolation of crystalline D-glucose and cellobiose from an enzymatic hydrolyzate of cellulose. J. Am. Chem. Soc., **75**, 1916–1918.

WHITAKER, D. R. 1953 Purification of *Myrothecium verrucaria* cellulase. Arch Biochem. and Biophys., **43**, 253–268.

WHITAKER, D. R. 1954 Mutarotation after hydrolysis of cellopentaose by *Myrothecium verrucaria* cellulase. Arch. Biochem. and Biophys., **53**, 436–438.

WHITAKER, D. R. 1954 Hydrolysis of a series of β 1.4 oligoglucosides by *Myrothecium verrucaria* cellulase. Arch. Biochem. and Biophys., **53**, 439–449.

WHITAKER, D. R., COLVIN, J. R., AND COOK, W. H. 1954 Molecular weight and shape of *Myrothecium verrucaria* cellulase. Arch. Biochem. and Biophys., **49(2)**, 257–262.